

arsonoacetic acids. Guanylic, uridylic and cytidylic acids were among many compounds found to have little or no effect.

7. Plant tissue L-glutamic acid decarboxylases (*Cucurbita*, *Daucus carota*) were found to behave like the bacterial enzymes with regard to their stimulation by phosphate, arsenate, nucleotides and pyridoxal phosphate.

8. Under similar conditions the activity of animal tissue decarboxylases (L-glutamic, cysteine, 3:4-dihydroxy-L-phenylalanine) were not increased by adenine nucleotides, other phosphates or arsenates.

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A Crystallographic Study of some Derivatives of Gramicidin S

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We first began the investigation of gramicidin S in 1944 at the suggestion of Dr R. L. M. Synge, to whom we are indebted for all the material used in the research. It appeared to us to be particularly interesting as a naturally occurring peptide of relatively low molecular weight, the study of which might throw light on the arrangement of amino acid residues in proteins. Further, it crystallized with surprising ease in crystals large enough for X-ray single-crystal analysis and it contains a functional group, the δ -amino group of ornithine, which made it possible to prepare a number of derivatives of the peptide, some containing heavy atoms. The present paper gives a survey of X-ray measurements on

several of these gramicidin S derivatives, undertaken as a preliminary to an attempt at a detailed X-ray analysis.

Gramicidin S was originally isolated in the form of a hydrochloride by Gause & Brazhnikova (1944). It has been shown by Synge and others (Consden, Gordon, Martin & Synge, 1947; Synge, 1948) to be a cyclic peptide built up from five different amino acid residues occurring in unimolecular proportions. These residues are L-valine, L-ornithine, L-leucine, D-phenylalanine and L-proline, written in the sequence in which they are combined in the molecule as indicated by the examination of partial hydrolysates.

When the peptide was first examined crystallographically, neither its composition nor its molecular weight was known, and our first aim was to derive from the X-ray measurements the molecular complexity of the peptide. This proved unexpectedly difficult. The peptide derivatives examined crystallize from ethanol or acetone which is present as solvent of crystallization in crystals immersed in their mother liquor, and a variable amount of this solvent is lost on exposure to the air. For this reason the mass of material present in the crystal asymmetric unit is never an exact multiple of the minimum pentapeptide unit + solvent. From the formal relations between the different crystal structures studied it is clear that the molecule itself can be either a pentapeptide or a decapeptide. The latter alternative appears to be established by the synthesis reported by Schwyzer & Sieber (1956); it is also supported by diffusion and cryoscopic measurements (Pedersen & Synge, 1948; Belozersky & Pashkina, 1945), by the countercurrent distribution of its 2:4-dinitrophenyl derivatives (Battersby & Craig, 1951), and by the apparent close relation between gramicidin S and tyrocidine A, which has been shown by Battersby & Craig (1952) to have the same 5 amino acid sequence as gramicidin S, together with five other amino acids.

The fact that gramicidin S crystallizes from volatile solvents present in small bulk made it difficult in some cases to prepare specimens for X-ray photography in the usual way by drawing crystals in their mother liquor into capillary tubes, since the solvent evaporated too rapidly. Suitable crystals were therefore generally grown by slow cooling from warm saturated solutions sealed in fine-walled Hysil glass capillaries and photographed *in situ*, surrounded by their mother liquor. Large single crystals, several millimetres long, were often obtained by this technique.

RESULTS AND DISCUSSION

Table 1 summarizes the preliminary X-ray data collected on twenty different crystalline forms shown by nine different derivatives of gramicidin S. These substances are (a) the hydrochloride, sulphate, hydriodide, flavianate, rufianate, and chloraurate; and (b) the *N*-acetyl, *N*-chloroacetyl, *N*-iodoacetyl derivatives, a molecular compound of the *N*-iodoacetyl derivative with iodine, and the *N*-(2:4-dinitrophenyl) derivative, prepared by Sanger (1946).

An appendix by Dr R. L. M. Synge provides chemical data on the different preparations

Table 1. *Data obtained by X-ray photography of single crystals of gramicidin S derivatives*

Derivative	<i>a</i>	<i>b</i>	<i>c</i>	β	Space group	<i>n</i> *	<i>p</i> †
Hydrochloride (1)	Dry		24.4	34.8	18.9	—	C222 ₁	8	2
(2)	Dry		24.9	34.2	37.7	—	C222 ₁	8	4
	Wet		26.1	35.3	37.9	—	C222 ₁	8	4
	Dry		24.1	34.6	18.9	—	C222	8	2
Sulphate	Wet		25.8	34.1	37.8	—	C222 ₁	8	4
	Dry		24.4	33.5	18.9	—	C222	8	2
Hydriodide (1)	Wet		28.0	41.1	39.2	—	I222 or I2 ₁ 2 ₁ 2 ₁	8	4
	Dry		22.3	41.8	39.6	—	I222 or I2 ₁ 2 ₁ 2 ₁	8	4
(2)	Wet		28.0	41.0	39.0	—	I222 or I2 ₁ 2 ₁ 2 ₁	8	4
	Dry		23.2	41.2	38.8	—	P2 ₁ 2 ₁ 2 ₁	4	8
(3)	Dry		23.0	72.8	19.8	—	P2 ₁ 2 ₁ 2 ₁	4	8
Flavianate	Dry		48.5	36.6	18.8	—	B22 ₁ 2	8	—
Rufianate	Wet		43	33	57	104°	C2	4	—
<i>N</i> -Acetyl	Wet		28.3	—	54.9	—	P6 ₁ 22	12	3
	Dry		27.3	—	55.2	—	P6 ₁ 22	12	3
<i>N</i> -Chloroacetyl	Wet		28.1	—	54.8	—	P6 ₁ 22	12	3
	Dry		27.5	—	54.8	—	P6 ₁ 22	12	3
<i>N</i> -Iodoacetyl	Wet		28.2	—	55.2	—	P6 ₁ 22	12	3
	Dry		27.7	—	54.7	—	P6 ₁ 22	12	3
<i>N</i> -Iodoacetyl + I ₂	Dry		24.4	40.5	38.5	—	I222 or I2 ₁ 2 ₁ 2 ₁	8	4
Chloraurate	Dry		21.1	38.5	40.5	—	P22 ₁ 2	4	8
	Dry		21.1	38.5	40.5	—	I222 or I2 ₁ 2 ₁ 2 ₁	8	4
<i>N</i> -2:4-Dinitrophenyl	—		31	34	19	—	—	—	—

* *n*, number of asymmetric units in the unit cell.

† *p*, number of pentapeptide units in the crystallographic asymmetric unit.

a, *b* and *c* are in Å.

examined. They dated from three different periods—1943, 1945–47 and 1952. While the earliest materials showed small differences in unit cell dimensions from those here recorded, probably owing to the presence of peptide mixtures, a sample of the iodo-acetyl derivative prepared from highly purified gramicidin S, specimen IV, examined in 1952, gave X-ray photographs identical with those obtained in 1945–47, showing that the small proportion of other peptide materials still present in the preparations at this date have no observable effect on the X-ray data.

Characteristics of wet and dry crystals of gramicidin S derivatives

Crystals of all the derivatives of gramicidin S that we have examined appear markedly birefringent and transparent and frequently show sharp, well-defined faces when covered with their mother liquor. On removal from this liquor they may become opaque and cracked, but slow drying often leaves them with both shape and transparency very little changed. X-ray photographs show that in all cases there are changes in the unit cell dimensions and in the relative intensities of certain of the X-ray reflexions. The reflecting power of the crystals diminishes on drying to a degree which depends on the rate of drying. Very slowly dried crystals show almost as many X-ray reflexions as wet crystals; these usually extend to spacings of approximately 1.5 Å.

Three types of change are observed during crystal drying, which are rather different from the changes observed with protein crystals. The most marked alterations occur with the hydrochloride and sulphate, where a change in crystal symmetry results in the halving of one unit cell dimension. All reflexions with l odd on the wet crystal photographs disappear, leaving the remaining reflexions comparatively little altered in position and intensity. The loss of solvent appears therefore to be accompanied by an adjustment of the positions of the molecules at z and $z + \frac{1}{2}$ in the wet crystal which renders them effectively identical in the dry crystal. Very little crystal shrinkage occurs but the changes in the molecular positions are not regular. Certain of the layer lines are markedly 'smeared' and diffuse reflexions appear, particularly from the hydrochloride. These phenomena are illustrated in Pl. 2*a* and *b*.

In the second type of change, that of the hydrides, the unit cells of wet and air-dry crystals remain of the same order of magnitude, but there is a marked shrinking in one direction, that of the a axis, of approximately 5 Å. This change suggests the existence of a single layer of solvent molecules which is removed on drying; it is similar to changes that occur on drying protein crystals, but is accom-

panied again, in one of the gramicidin S hydride crystals, by a change of crystal symmetry.

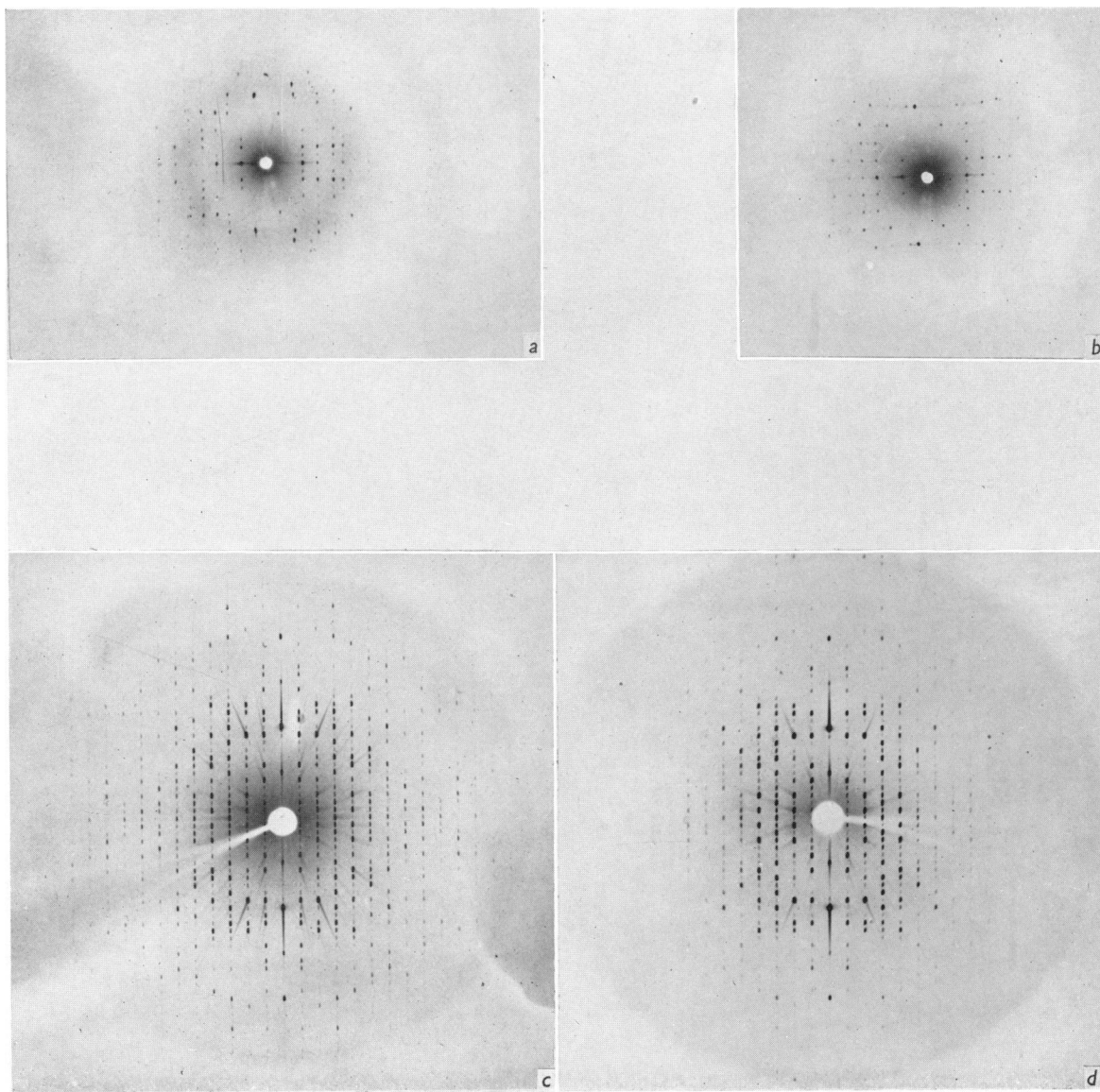
In the third type of change, that of the acetyl derivatives, there is very little alteration in the unit cell dimensions. There are, however, small changes in the intensities of some of the X-ray reflexions which indicate that here the solvent molecules are zeolitic, able to pass out of the lattice, leaving the position and orientation of the peptide units practically unchanged. The order of magnitude of the solvent loss on drying is illustrated by measurements by Dr Synge: he observed that a preparation of wet crystals of acetylgramicidin S from which all visible drops of water had been removed weighed 55 mg. After being dried in a vacuum desiccator at room temperature, it weighed 40.5 mg.; this weight increased to 43.3 mg. on exposure to the air.

Molecular weight of gramicidin S

The stoichiometric unit. The determination of the minimum stoichiometric molecular weight from the X-ray data depends on the accuracy with which the cell dimensions, density and solvent content of the crystals can be measured. So far no attempt has been made to measure the solvent content of the wet crystals, and the estimations are therefore derived from the air-dry crystals.

There are experimental difficulties in measuring both the cell dimensions and the density with high accuracy. The number of observed reflexions is smaller for some of the air-dry crystals than for the wet crystals, and these are often not sharp, owing to the disordered character of the dry crystals. The density measured by flotation in aqueous salt solutions is found to vary with the time of immersion of the crystals, presumably owing to solution entering the crystal lattice. For each crystal studied, the value for the density recorded in Table 2 is that observed for freshly wetted crystals and should certainly be regarded as a maximum; some solvent molecules may enter the crystals directly they are immersed. But it is probable that the main difficulty in obtaining a measure of the peptide molecular weight from X-ray data lies in the accurate determination of the solvent content of the air-dried crystals. This has usually depended on a measurement of solvent loss on drying *in vacuo* at 100°, followed by analysis of the dried crystals.

With gramicidin S crystals we observed a serious discrepancy at the beginning of our research with the hydrochloride, which is recorded in Table 2. The figure for the weight of the crystal unit less solvent lost on drying at 100° is very much higher than the chemically determined weight of a pentapeptide unit, even if this is associated with one molecule of water of crystallization as suggested by the analytical data on the vacuum-dried crystal (Table 2). It seems clear that the air-dried crystals contain



(a) Oscillation photograph about the *c*-axis of wet gramicidin S sulphate. (b) Oscillation photograph about the *c*-axis of dry gramicidin S sulphate. (c) Precession photograph of dry *N*-chloroacetylgramicidin S, showing (0*kl*) reflexions. (d) Precession photograph of dry *N*-acetylgramicidin S, showing (0*kl*) reflexions.

G. M. J. SCHMIDT, DOROTHY CROWFOOT HODGKIN AND BERYL M. OUGHTON—
A CRYSTALLOGRAPHIC STUDY OF SOME DERIVATIVES OF GRAMICIDIN S

Table 2a. *Molecular-weight data on gramicidin S hydrochloride*

Suggested formula of vacuum-dried crystals	Loss of wt. on drying at 100°	Mol.wt. of suggested formula	Minimum immersion density	X-ray mol.wt.
$C_{30}H_{46}O_6N_6 \cdot HCl \cdot H_2O$	6.1 %	624	1.217	687 \times 2

Table 2b. *Molecular-weight data on N-acetylgramicidin S and its substitution products*

Derivative	Suggested formula of air-dried crystals	Mol.wt. of suggested formula	Minimum immersion density	X-ray mol.wt.
N-Acetylgramicidin S	$C_{32}H_{48}O_6N_6 \cdot C_2H_5 \cdot OH \cdot 1.5H_2O$	685	1.165	687 \times 3
N-Chloroacetyl-gramicidin S	$C_{32}H_{47}O_6N_6Cl \cdot C_2H_5 \cdot OH \cdot 1.5H_2O$	719.5	1.238	742 \times 3
N-Iodoacetyl-gramicidin S	$C_{32}H_{47}O_6N_6Cl(0.2)I(0.8) \cdot C_2H_5 \cdot OH \cdot 1.5H_2O$	793	1.345	816 \times 3

more solvent than the analytical data suggest. Some attempt was made to check this idea by having the chemical analyses for chloroacetyl- and iodoacetyl-gramicidin S carried out on the air-dried instead of on vacuum-dried crystals. The analytical figures suggest that both ethanol and water are present in amounts which give much better agreement with the conclusions from X-ray data. Within the limits of error of both types of measurement it seems likely that the formula unit in the acetyl series of crystals approximate to 1 molecule of pentapeptide:1 molecule of ethanol:1.5 molecules of water, and this is shown in Table 2. But it should be realized that in crystals which are disordered to some extent the solvent molecules may well be present in fractional amounts as suggested in the analytical description in Appendix 1, and until a detailed X-ray analysis of the crystals can be carried out our knowledge of the contents of the crystals will remain rather imprecise.

Our attitude to our observations is naturally affected by our knowledge of the chemical constitution of gramicidin S. Our experience indicates that when X-ray methods are used to determine unknown molecular weights of this order of magnitude the errors most likely to occur may increase the estimated molecular weight by as much as 10 %.

The absolute molecular weight. The smallest molecular-weight unit that appears as the asymmetric unit (the simplest repeating unit) in any of the crystal structures examined is the unit corresponding to a decapeptide, which is observed in the air-dry forms of gramicidin S sulphate and hydrochloride. However, in the hexagonal acetylgramicidin S series, the asymmetric unit contains three pentapeptide units. The first, most natural, deduction from these two observations is that the molecule itself may be a pentapeptide, which owing to complexities of packing sometimes occurs in two positions not related by the space-group symmetry of the crystal, and sometimes in three. But there is

an alternative formal possibility, which is most likely. In the hexagonal space group $P6_322$ the two-fold axes of symmetry present can be symmetry elements of the molecules themselves. In this case, the crystal asymmetric unit may be essentially three half decapeptide molecules, or $1\frac{1}{2}$ molecules, provided that the decapeptide molecule itself has a twofold axis of symmetry. Formally, therefore, the crystallographic evidence requires the molecule to be either a pentapeptide or a decapeptide having a twofold axis of symmetry. The decapeptide structure alone is in accord with its chemical synthesis and with the measurements carried out by Pedersen & Synge (1948) and by Battersby & Craig (1951).

Size and shape of the molecules

While it is not possible to make precise deductions about the size and shape of a molecule of the complexity of gramicidin S from our present data, there are certain features of these data which are suggestive and worth further comment.

Throughout the series of crystal structures one unit cell dimension (usually c) is 18.8–19.8 Å, or some multiple of this figure. In this direction a very strong reflexion, (004) in the hydrochloride, and (000,12) in the acetyl series, shows that many atoms are concentrated in layers about 4.8 Å apart. Pl. 2c, d illustrates the overwhelming strength of the (000,12) reflexion in *N*-chloroacetyl- and *N*-acetylgramicidin S. The formal consequences of this are illustrated in Fig. 1a by the section at $x=0$ in the three-dimensional Patterson series for *N*-acetylgramicidin S. The strong peak (A) shows that there are many vectors parallel to c between atoms 4.8 Å apart. The marked streaking along lines parallel to a suggests that these atoms are in layers.

The remaining two unit-cell dimensions, a and b , vary in their ratio to one another in the different crystal structures, but a close relationship can again be traced between the orthorhombic and hexagonal

structures; the conditions limiting the molecular size can best be seen from the latter type of structures. In Fig. 1*b* is shown the section of the *N*-acetylgramicidin S Patterson series calculated at $z = 0$. This indicates that the whole crystal structure repeats approximately at the positions $\frac{1}{2}a_1$, $\frac{2}{3}a_2$ and $\frac{2}{3}a_1$, $\frac{1}{3}a_2$. The character of the vector distribution is best understood if the molecules are rather compact. They need not be cylindrical but could have an elliptical or an approximately rectangular cross-section; if so, extreme values for the two sides of the rectangle might be set at about 11 and 19 Å. A decapeptide molecule having these dimensions would extend about 9.5 Å, or $c/6$, in the c direction. Thus, within the molecule would be two layers of atoms approximately 4.8 Å apart.

Detailed X-ray analysis of gramicidin S

Of the different crystal structures so far examined those of *N*-acetyl-, *N*-chloroacetyl- and *N*-iodo-

acetyl-gramicidin S appear most suitable for use in attempting a detailed X-ray analysis. The compounds are very nearly isomorphous and, as Pl. 2*c* and *d* shows, some of the X-ray reflexions from corresponding crystal planes show considerable changes in intensity, from which the positions of the heavy atoms may be found and the phases of the reflexions subsequently inferred. The molecular arrangement is very complicated, but nevertheless presents a far simpler X-ray analytical problem than do the simpler proteins. We are accordingly collecting three-dimensional X-ray data from these crystals and attempting to find a detailed solution of their structure.

CONCLUSION

More definite crystallographic knowledge of the character of the gramicidin S molecule must await the outcome of this detailed study. But already in this preliminary survey of evidence concerning the molecular weight, size and shape we have made certain observations which bear on the investigation of proteins. Our experiences in the molecular-weight determination of gramicidin S can, for example, be paralleled by measurements on insulin. It is noticeable that the best crystallographic value for the molecular weight of zinc insulin, 11 900, is numerically larger than the comparable figure, 11 554, derived by calculation from the amino acid composition; and this figure, 11 554, again is twice the actual minimum chemical stoichiometric figure of 5777 (Sanger & Tuppy, 1951; Sanger & Thompson, 1953). This suggests that in insulin crystals, as in gramicidin S crystals, molecules may be fitted together in the crystal asymmetric unit in ways which are not governed by the symmetry operations of the crystal. Lastly our present evidence on the size, shape and symmetry of the gramicidin S molecule limits considerably the way in which the amino acid residues can be arranged within the molecule and has led us naturally to build a number of possible model structures. These structures appear to us to have interesting and conflicting relations with present theories of peptide-chain configurations in proteins. Since it may still be years before we can solve the detailed structure of gramicidin S we have described in Appendix 2 the models we have so far constructed and their relation to ideas of protein structure.

EXPERIMENTAL

The main practical problem in this work was the photography of crystals in the wet state. The method usually employed with crystalline proteins of drawing up the crystal into a capillary tube from its aqueous mother liquor proved impracticable, particularly so with the hydrochloride. Here slow evaporation of a concentrated solution in 75% (v/v) aqueous ethanol resulted in a mush of small

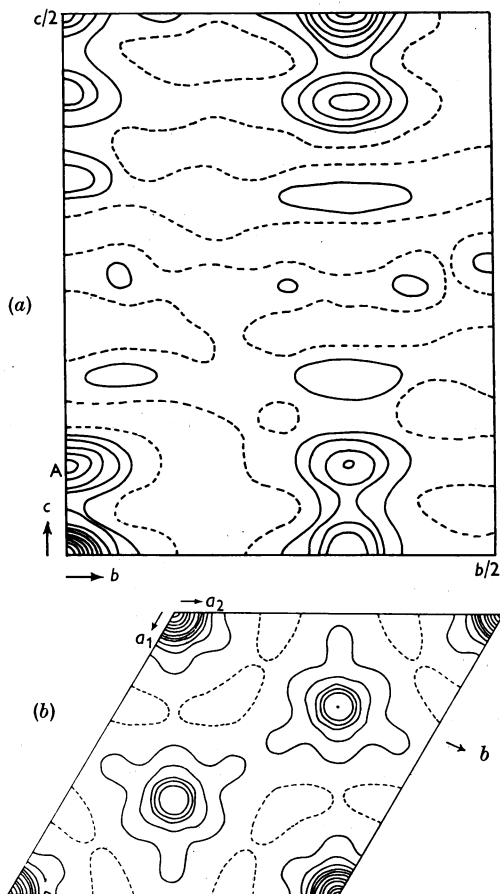


Fig. 1. Sections from the 3-dimensional Patterson synthesis for *N*-acetylgramicidin S at (a) $x, 2x, z$; (b) $z = 0$.

crystals; the solvent evaporated before a single crystal could be sorted out or introduced into the capillary. This difficulty was overcome by growing single crystals in thin-walled capillaries so that they could be photographed *in situ*. In this way large crystals were produced from 50 mm.³ of solution or less, i.e. recrystallization could be carried out on a micro scale.

The capillaries were blown from Hysil glass with an average diameter of 1 mm. and an average wall thickness of 20 μ . Hysil was chosen in preference to Lindemann glass because of the low pH of the solution. Hysil contains no Fe, and its constituents are of relatively low atomic weight. A suitable quantity of liquid (usually a column about 5 mm. long) with a small amount of solid was drawn up into the capillary which was then sealed at both ends in the micro-flame. The capillaries were immersed in a large, well insulated tank containing water whose temperature was controlled by an electric heating element with variable energy input. No precautions were taken to ensure a perfectly uniform temperature drop, but recrystallization was usually successful on cooling from about 75° to room temp. at an overall rate of 1° in 4 hr., and in favourable cases gave crystals up to 5 mm. long. The method has so far not been successful with the flavianate and the 2:4-dinitrophenyl derivative.

To compare wet with dry crystals our method at this stage was simply to break the capillary, drain the liquid by inserting a smaller capillary, and allow the crystal to dry slowly.

Copper radiation ($\lambda = 1.542 \text{ \AA}$) was used for all the X-ray photography. Most photographs were taken on flat films with a crystal-to-plate distance of 4.0 cm.; with a pinhole slit system (second aperture 0.4 mm.) very clean photographs were obtained after exposures up to 48 hr. without special precautions. Cell dimensions were calculated either from layer line measurements on cylindrical films of 3 cm. radius, or from symmetrical photographs recording several orders of, say, ($h00$) and ($h00$), taken for the most part on X-ray plates to avoid shrinkage errors. We cannot claim accuracy in cell dimensions greater than 2%. A few recent photographs were taken on a precession camera.

Gramicidin S hydrochloride

Gramicidin S HCl crystallizes very readily in small needles from aqueous ethanol; larger crystals were grown by slow cooling from 70% (v/v) aqueous ethanol. The crystals are elongated along [001], showing {110} and {001}.

$$\alpha \parallel a, \beta \parallel c, \gamma \parallel b.$$

The data given in Table I refer to two different batches, of which the first was less pure than the second. This first batch contained dry crystals of two kinds which gave different X-ray photographs, and are listed as (1) and (2). It is noticeable that the *c*-axis dimension of (2) is similar to that of the wet crystals of later preparations; probably they contained impurities which slowed down the normally rapid transformation of wet to dry crystal structures.

The stable dry forms of the two batches are very similar in cell dimensions, intensities of X-ray reflexions and the types of diffuse reflexions shown in X-ray photographs, i.e. particularly marked smear lines running through reflexions of index ($hk4$). One crystal photographed from the second batch showed unusual diffuse reflexions which indexed on a body-centred lattice with a doubled *c* axis.

Gramicidin S sulphate

The sulphate resembles the hydrochloride extremely closely. The crystals are needles, elongated along [001], showing {110} and {001}, also thin plates on {010}, showing {100} and {001}.

$$\alpha \parallel a, \beta \parallel c, \gamma \parallel b.$$

On drying, the crystals show disorder phenomena as with the hydrochloride. Marked smear lines appear on photographs through reflexions of index ($hk4$); on some photographs diffuse reflexions occur requiring a doubled and trebled *a* axis.

Gramicidin S hydriodide

Three forms were observed, of which one was prepared by Syngé. The other two occurred in a preparation made at Oxford, which was slightly discoloured, suggesting the presence of some free iodine. Unfortunately no analytical data were obtainable.

Both modifications (1) and (2) were well-developed needles elongated along [001] and showing {110} and a variety of unidentified domal faces. Optically, both forms showed

$$\alpha \parallel a, \beta \parallel c, \gamma \parallel b$$

when wet; drying produced marked loss of birefringence, accompanied perhaps by a change in optical orientation.

Crystals of the third form are small, clear plates on {010}, found in one batch of Oxford crystals. They gave unusually good X-ray photographs, showing no sign of crystal disorder.

Gramicidin S flavianate

The flavianate has not been photographed in the wet state, since all attempts at recrystallization in tubes produced bunches of needles. The crystals are elongated along [001] and show {100} and {010};

$$\alpha \parallel c, \beta \parallel a, \gamma \parallel b.$$

The natural interpretation of the X-ray data on this crystal is that the unit cell contains sixteen decapeptide molecules and sixteen flavianic acid molecules. However, most preparations have shown, on analysis, higher contents of flavianic acid than this. Possibly some flavianic acid replaces gramicidin S at random in the lattice.

Gramicidin S rufanate

The rufanate was prepared at Oxford by mixing solutions of 2 mg. of gramicidin S HCl and 2 mg. of rufanic acid in absolute ethanol. The dark-red crystalline precipitate formed was filtered off, washed with 70% ethanol and recrystallized once from absolute ethanol. Large crystals, which were red needles, elongated along [010], were grown by slow cooling from 90% (v/v) aqueous ethanol.

On drying, the crystals lose their birefringence and become opaque; X-ray photography shows nearly complete breakdown of the crystal lattice.

N-Acetyl-, N-chloroacetyl- and N-iodoacetyl-gramicidin S

These three acyl derivatives are nearly isomorphous. They occur in beautifully-formed hexagonal needles showing {10 $\bar{1}$ 0} and {10 $\bar{1}$ 1}; occasionally also bipyramids with only {10 $\bar{1}$ 1} are observed. They are optically negative. Very slow drying yields crystals with clear faces which give good measurable reflexions in the optical goniometer. In general

the pattern of the intensities of the X-ray reflexions is very similar in the three crystals, but some changes are observable both on drying and on halogen substitution. Analysis shows (see Appendix 1) that there is incomplete substitution of the Cl by I in the *N*-iodoacetyl derivative.

Iodoacetylgramicidin S crystallized in the presence of excess of iodine

An orthorhombic form of iodoacetylgramicidin S was obtained by Synge in 1952, by crystallizing it from aqueous ethanol in the presence of excess of iodine. Beautiful little brownish-red needle-shaped crystals were formed, elongated along [010] with well-developed {100} and other unidentified domal faces. These showed marked pleochroism, from which it could be deduced that the iodine molecules lay perpendicular to the needle axis. Unlike most of the derivatives examined, the needle axis is not the one which is a multiple of (18.8–19.8 Å), but the optics seem to bear the same relationship to the cell dimensions as in other crystals. $\gamma \parallel b$, and probably $\alpha \parallel a$ and $\beta \parallel c$.

Gramicidin S chloroaurate

The chloroaurate was prepared by Synge in 1952, and grows in the form of colourless, flat needles, elongated along [001], with well developed {100} or {010}.

Of several crystals selected, one was found to have crystallized in a different space group from the others. All of them had the same unit cell dimensions, and most seem to be body-centred, probably having the space group I 222; the one exception had the space group P2₁2.

The crystals were weakly birefringent, with $\alpha \parallel a$, $\beta \parallel c$ and $\gamma \parallel b$.

SUMMARY

1. Single crystals of a series of derivatives of gramicidin S have been prepared and examined by X-ray methods. Their morphology, unit cells and space groups have been determined, and a comparison between crystals in the wet and dry states has been made.

2. The weights of the crystal asymmetric units of the hydrochloride and *N*-acetyl, *N*-chloroacetyl and *N*-iodoacetyl derivatives have been determined by X-ray methods and the results compared with the molecular weights determined by chemical analysis.

3. It is concluded that the gramicidin S molecule is most probably a decapeptide in which two halves of the molecule are related by a twofold axis of symmetry. Some conclusions about the size and shape of the molecules are drawn.

4. Appendix 1 describes the methods of preparation and chemical analyses of the crystals examined.

5. In Appendix 2 possible molecular models for gramicidin S are discussed in relation to current theories of protein structure.

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APPENDIX 1

Preparation of some Derivatives of Gramicidin S

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N (Kjeldahl) determinations were done by the incineration procedure of Campbell & Hanna (1937). Other elementary analyses, unless otherwise stated, were by Weiler and Strauss, Oxford. Evaporations, unless otherwise stated, were done *in vacuo* below 40°.

Starting material: gramicidin S hydrochloride. Specimens I and III (Synge, 1945; Consden, Gordon, Martin & Synge, 1947) were first sent for crystallographic study and used for preparing derivatives. Later preparations have all been crystallized from the same batch of crude material as specimen III. One such preparation was studied by counter-current distribution by Dr Lyman C. Craig and colleagues

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